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**Geothermal Environmental Impact Assessment
Procedures for Using Fauna as Biological Monitors of
Potential Geothermal Pollutants**

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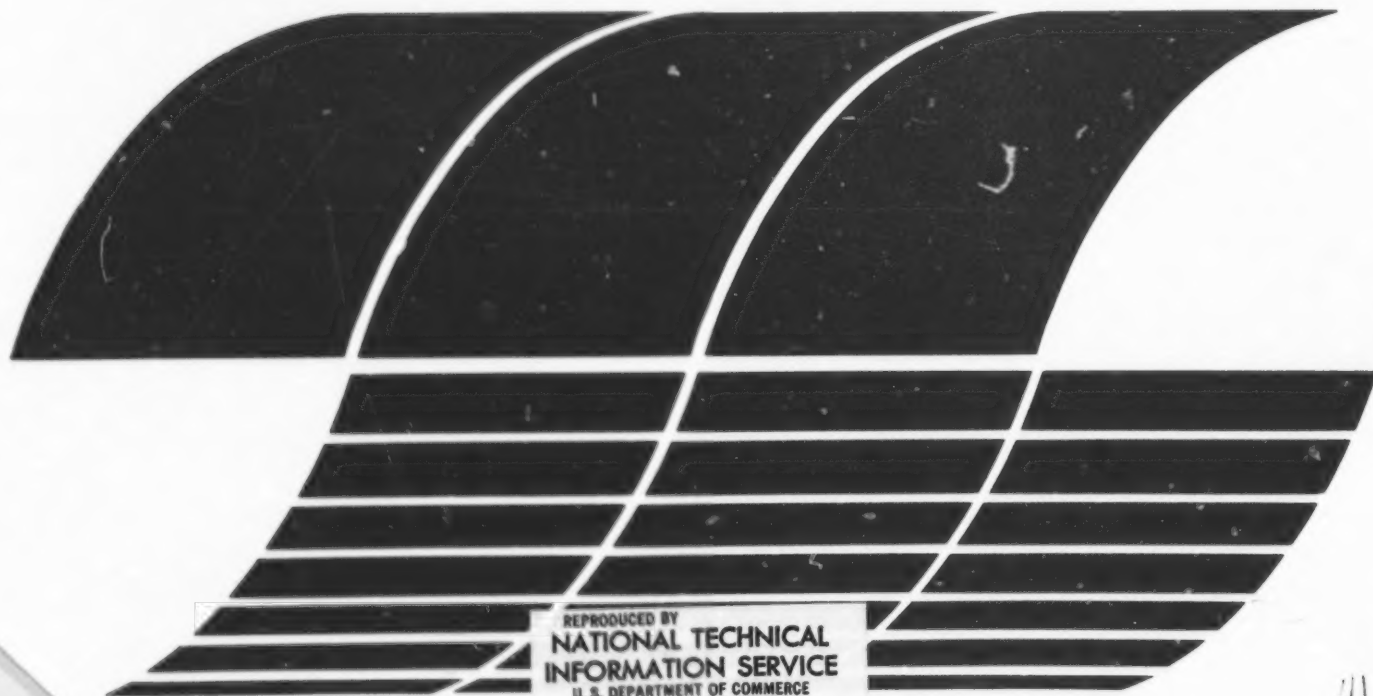


Geothermal Environmental Impact Assessment:

Procedures for Using Fauna as Biological Monitors of Potential Geothermal Pollutants

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GEOTHERMAL ENVIRONMENTAL IMPACT ASSESSMENT

Procedures for Using Fauna as Biological Monitors of Potential
Geothermal Pollutants

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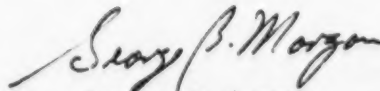
FOREWORD

Protection of the environment requires effective regulatory actions which are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specific pollutants in the environment requires a total systems approach which transcends the media of air, water, and land. The Environmental Monitoring and Support Laboratory-Las Vegas contributes to the formation and enhancement of a sound integrated monitoring data base through multidisciplinary, multimedia programs designed to:

develop and optimize systems and strategies for monitoring pollutants and their impact on the environment

demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report presents preliminary data on trace element tissue levels, population characteristics of small mammals and discusses analytical and field methodology for determining the feasibility of using livestock and domestic animals as biological monitors of potential geothermal pollutants. This report is part of an overall program which will combine data on air, water, soil, flora and fauna in assessing the environmental impact and design of a monitoring strategy for geothermal resource development. For further information the reader should contact the Monitoring Systems Exposure Dose Assessment Branch (MSE).



George B. Morgan
Director

Environmental Monitoring and Support Laboratory
Las Vegas

ABSTRACT

This is the first in a series of reports that covers the feasibility of utilizing wildlife and domestic animals to design a strategy for assessing the environmental impact of geothermal resource development. This study is part of an overall program which will also include data on air, water, soil and flora.

Animal tissues and animal products were collected in the vicinity of California and Utah geothermal development sites. These samples are being assayed for such elements as lead, cadmium, zinc, boron, aluminum and strontium so as to confirm baseline concentrations in the tissues of area fauna. Selected samples were analyzed by atomic absorption but most of the analyses were by optical emission spectrometer, which required freeze-dried, homogeneously mixed samples. Small mammal population characteristics are being monitored at Roosevelt Hot Springs, Utah. Those animal species sampled in the field are being studied under controlled conditions to relate the ingestion of selected elements to subsequent changes in the elemental concentration of various tissues.

This report presents some preliminary data on trace element concentrations in tissues of wildlife and domestic animals. Concentrations in geothermal effluents also were determined. Quality assurance, sample collection, sample preparation, analytical procedures, and relative abundance of small mammals are discussed.

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INTRODUCTION

The encouraging ecological feature of geothermal energy is that it is basically a contained system. The effluent rises to a heat exchanger and, in many cases, would be reinjected down a second well without exit to the surface atmosphere. Environmental damage therefore should be slight. Nonetheless, trace amounts of toxic elements frequently precipitate out of steam condensates and under some conditions might be transported to surface waters or dispersed as aerosols. The effect of geothermal development on area animal life is largely unknown. The delicate nature that comprises a native environment can be altered by changes in the elemental flow through an ecosystem, by destruction of habitats, human presence and activity, etc. (Berman, 1975). To assess any possible impact and to help design a monitoring strategy for geothermal development, baseline elemental tissue levels are being collected and population parameters are being monitored.

This report is the first in a series that will lead to the development of a strategy for monitoring any pollutants to which people might be exposed as a result of geothermal generation. An EMSI.-LV team has been investigating the use of wildlife and livestock as biologic monitors and presents herewith a progress report on the first year of research.

The major contribution thus far is the evolution of procedures for determining levels of trace elements in fauna and wildlife population studies, with the emphasis on census, food habits and habitat associations.

The field work was done at geothermal project sites in the Imperial Valley of California and at Roosevelt Hot Springs in Utah. Trace element and census data appear here in their most preliminary form. Later reports on faunal monitors will fill in the gaps, with a view to establishing baseline data, on elements and population, against which later surveys, reflecting the impact of the power plants, can be compared. The objective is to set the baselines before industrial development alters the indigenous picture. However, as this manuscript is written, the fauna team has made significant progress on quality procedures for trace element analysis and on small mammal population surveys.

Animal tissues and products have been collected at both the California and Utah sites and are undergoing assay in the effort to establish baseline concentrations of elements, such as lead, cadmium, zinc, boron, aluminum, arsenic and strontium. Small mammal population is being monitored at the Utah site.

TRACE ELEMENT PROCEDURES

TISSUE COLLECTION

Biological sampling has been conducted at Imperial Valley on a quarterly basis. These collections consist of primarily cattle hair and blood with a limited number of poultry samples. Resident waterfowl, fish and occasional honey samples have also been collected on an intermittent basis. Since new cattle are continually being delivered to Imperial Valley the traditional concept of baseline sampling has included cattle recently delivered to the Imperial Valley feedlots. Poultry samples were included in the collection effort because the birds were resident animals. It is expected that dietary variations in selenium, fluorine, manganese and mercury should be correlated with either the amount of the element in the whole egg or its distribution between yolk and egg white.

The California collections have also included samples from a locally nesting water bird, the American Coot (*Fulica americana*). Samples of muscle, sternum, rib, femur, liver, spleen, kidney, lung, claws, and feathers have been taken from a few birds. Coots are omnivorous and much of their food is obtained from, or near, the water where aquatic vegetation, aquatic insects, crustaceans, and small fish contribute to their diet. However, flocks of coots are occasionally sighted on cultivated fields near a geothermal test facility.

Collections at Roosevelt Hot Springs, Utah have been made on a seasonal basis to acquire wildlife samples and twice yearly for domestic animal samples. Samples of hair/wool and blood from sheep and cattle were made in the spring and fall, seasons that coincided with the roundup and movement of animals between winter and summer ranges. Rodents were collected in museum special snap traps, and roadside kills were taken in the case of rabbit collections. Tissues or organs taken from each animal have included bone, hair, lung, liver, kidney and in some cases blood.

Cattle and sheep samples were sequentially numbered during each collection period and identified with the date, livestock owner and location of the feedlot or grazing allotment area. The location, approximate size and total grazing time for both summer and winter ranges were obtained for Utah livestock and, when possible, the geographic origin and time of arrival was acquired for cattle in the California feedlots. Poultry samples were also numbered and identified as to owner, location and collection date. Rodents and lagomorphs were identified (species), weighed, sexed and, during the more recent trapping periods, selected body measurements and the reproductive condition were recorded. During the respective collection efforts, hair or

wool was clipped from the animal's side and sealed in polyethylene bags. Blood samples were collected by jugular venipuncture in heparinized syringes or in heparinized vacutainer tubes and wildlife carcasses were sealed in polyethylene bags. All samples were transported back to Las Vegas in ice-filled cooler chests.

Muscle and fat were scraped from the bone samples and the hair samples were cleaned by the method of Clarke and Wilson (1974). Surface contamination was removed by washing the hair in separate solutions of soap, acetone and ethylenediaminetetraacetic acid (EDTA). Wet weights were recorded for all sample types and the collected material was either freeze-dried (blood, egg white and yolk, and some rabbit tissues) or oven-dried (rodent tissue, all hair and bone samples) at 60°C. Dry weights were recorded and the individual samples transferred to acid washed polyethylene vials and stored under refrigerated conditions. The dried samples were subsequently ground with a cryogenic mill or manually mixed with a Teflon spatula or an agate mortar and pestle. All laboratory ware, mostly polyethylene, was washed with dilute nitric acid and followed by a deionized water wash. Stainless steel, polyethylene and Teflon spatulas, surgical instruments, etc. were cleaned in a similar fashion. Detailed procedures on sample preparation are described in Appendix A and B.

LABORATORY ANALYSIS AND QUALITY ASSURANCE

Flame and flameless atomic absorption spectrophotometry, optical emission spectroscopy and x-ray spectrometry have been used at EMSL-LV for various projects involving elemental analysis. Additional techniques include neutron activation, spark source mass spectrometry, pulse polarographic methods and anodic stripping voltammetry. A summary comparison of these methods has been presented by Lisk (1974). In the current study, DC arc optical emission spectroscopy and flame atomic absorption spectrophotometry were the analytical tools utilized.

Optical emission spectroscopy has a simultaneous multi-element capability. The technique is precise, rapid, and adaptable for solid or liquid samples. The sample is vaporized, usually via electrical discharge, and a fraction of the atoms are excited to unstable energy levels. When the atoms revert to their stable states, they emit the absorbed energy as characteristic optical spectra. A spectrograph disperses the emitted radiation and individual detectors record the intensities of the spectral lines of interest. These intensities are related to the concentrations of the elements in the sample. In our case, 26 elements may be detected simultaneously through standard DC arc optical emission techniques. The relative standard deviation of this analytical procedure has been reported at between 3 and 15 percent for finely ground material with acceptable accuracy above 1 µg/g for most elements (Alexander et al., 1975).

When solid samples are analyzed in this way, homogeneity is a prerequisite since non-homogeneous elemental distribution results in interpretation errors that cannot be completely eliminated by replicate analyses. An effi-

cient sample-mixing process is therefore essential, and for our determinations a cryogenic Tekmar mill (stainless steel chamber and carbide cutting blades) served well. Liquid standards for optical emission spectroscopy are relatively easy to prepare and use. However, acceptable synthetic standards are very difficult to prepare from undigested ground material with a uniform elemental distribution. Dried tissue standards of known elemental concentrations were therefore acquired from the National Bureau of Standards, and both subsamples and field-collected material have been routinely analyzed. Furthermore, a collection of eggs, blood, and liver have been freeze-dried and stored for repeated assays. The Laboratory of Nuclear Medicine and Radiation Biology, UCLA conducted three separate analyses on all samples and standards.

Atomic absorption spectrophotometry is a fast, sensitive, and precise analytical technique; however, it does not have a simultaneous multi-element capability. A hollow-cathode lamp (one for each element to be analyzed) emits light of the characteristic frequencies of the element of interest. This light passes through a flame (acetylene or hydrogen plus air or nitrous oxide) into which the sample solution is introduced by a nebulizer and the sample atom, excited by the flame, absorbs part of the radiation emitted by the hollow-cathode lamp. The resulting weakened light beam goes through a high resolution spectrometer. The isolated line, characteristic for the element under investigation, is directed to a photomultiplier tube where the light is transformed to an amplified electric signal. About 70 metallic elements can be determined by atomic absorption spectrophotometry.

The samples to be analyzed must be in solution form, meaning tissue samples must be digested. This handling increases the possibility of contamination and partial loss of sample constituents. Samples undergo wet oxidation prior to atomic absorption assays. The vast majority of wet oxidation procedures use some combination of four reagents: sulfuric acid, nitric acid, perchloric acid and hydrogen peroxide. We chose nitric acid since it has been widespread in its usage. The initial nitric acid digestions conducted for this study were time consuming and therefore, a commercial microwave oven has been adapted (Abu - Samra et al., 1975) as a faster alternative. Oven adaptations included an internal Pyrex liner, exhaust openings at the rear of the oven and an attached scrubber and exhaust fan to control the acid fumes. Details of the procedure are given in Appendix I.

Because the material to be analyzed is in solution, the preparation of standards presents few problems. From EMSL-Cincinnati, standard quality control solutions have been obtained which contain 15 elements at $\mu\text{g/l}$ concentrations and which are suitable for dilution to the required concentrations. In our work duplicate determinations are usually conducted and the linearity, which is assured by instrument components, of the calibration curve (absorbance versus concentration) is checked and recorded daily. A blank or solvent is analyzed between each set of 10 samples or standards to verify baseline stability. These control solutions do not contain sample residues, but do contain the same digestion reagent residues present in the regular samples. A few samples were spiked at different concentrations after sample digestion so that an analytical recovery estimate could be made. At present atomic absorption analyses are in initial stages and only preliminary

unevaluated data for arsenic have been obtained. Future analyses will include other elements and use of standards from EMSL-Cincinnati. The effect of storage time, container type and grinding technique is being determined with spiked and unspiked samples. Nonetheless, when sample preparation and analysis time and overall reagent costs are considered, emission spectroscopy appears preferable for most routine assays.

Two additional studies have been initiated as part of this program. Those animal species sampled in the field will be studied under controlled conditions in an attempt to relate the ingestion of selected elements to subsequent changes in the elemental concentration of various tissues. Pre- and post-exposure tissue concentrations will be emphasized. Rodents, rabbits, chickens, sheep and beef cattle are major representative species in the field sampling program and have been the main candidate animals for this experimental series. During 1977 rodents and rabbits, initiating the series, were administered either oral doses of concentrated geothermal effluents or, in some cases, single elements of particular interest. The rodents had been collected in the field, but domestic rabbits were substituted for field lagomorphs because jackrabbits are difficult to capture and use under laboratory conditions. The animals were serially sacrificed, for tissue collection, following acute or chronic exposures, as summarized in Table 1. The results, when completed, will help determine which tissues and/or bodily divisions might best reflect environmental exposure. Because of interrelated work, currently in progress through a grant managed by EMSL-LV, as well as numerous publications on some of the elements, these laboratory studies have not been extensive. However, the experiments have been an essential part of the quality assurance effort inasmuch as the laboratory-generated samples have been analyzed by the same techniques as those used for the field-collected tissues.

CENSUS PROCEDURES

The relative abundance of lagomorphs was determined through nighttime observations, and all rabbits and hares visible from the road by spotlight were recorded. The data are presented as actual counts per kilometer travelled.

Live traps, baited with rolled oats and placed along census lines, were used to collect rodents for identification and determination of their relative abundance at Roosevelt Hot Springs. Two parallel lines were laid out 50 meters apart and 240 meters long each containing 25 Sherman traps at 10-meter intervals. Two sets of parallel census lines were set at each location yielding 100 traps. One pair of such lines was 200 meters west of a primary drilling site and another was about four miles south. Trapping went on for two days and three nights and, following trap inspection, each captured animal was identified, sexed, examined for reproductive condition, marked by toe clipping and released. Results on relative abundance were based on the number of recaptures and presented as the expected population (Lincoln, 1930; Cockrum, 1962). A similar trapping program is to begin shortly in the Imperial Valley, California.

TABLE 1. OUTLINE OF LABORATORY STUDIES CONDUCTED TO DETERMINE ELEMENTAL CONCENTRATIONS
IN TISSUES OF SMALL MAMMALS AT ROOSEVELT HOT SPRINGS

Animal	No. of Orally Dosed Animals	Dosing Material	No. of Doses	Cumulative No. of Dose per Animal	Number and Types of Samples				
					Animals	Blood	Liver	Kidney	Bone Hair
Desert woodrat	9	As ₂ O ₃	3	600 µg	8	17	17	17	17 9
Desert woodrat	9	CdCl ₂	3	600 µg	8	17	17	17	17 9
Domestic rabbit	12	As ₂ O ₃ ge*	3	3.33 mg 1.33 ml	8	20	20	20	20 20
					Unwashed Gastrointestinal Tract				
Desert woodrat	9	CdCl ₂	3	1.20 mg	7	16	16	16	16
Ord's kangaroo rat	6	As ₂ O ₃ ge*	10	1.50 mg 2.5 ml	4	10	10	10	10
Laboratory rat	6	As ₂ O ₃ ge*	10	1.50 mg 2.5 ml	7	13	13	13	13

* In these experiments the arsenic dose was added to geothermal effluent, and the amount of arsenic spike as well as the volume of effluent administered per animal is shown.

PILOT PROJECT

STUDY AREAS

Imperial Valley encompasses about 1,700 square miles including 500,000 acres of irrigated farm land where crops are produced throughout the year. Major vegetable crops include lettuce, onions, melons, tomatoes, and carrots, while alfalfa, wheat, sugar beets, and cotton are among the prominent field crops. The valley is also a major cattle-feeding area. Many of the beef animals are transported great distances to the Imperial Valley feedlots from Texas, Mississippi or Louisiana. Cattle arrive weighing about 400 pounds and are processed initially in "calf lots" before transfer to a main lot or leased pasture plots. One complicating factor in feedlot sampling is that these animals may graze periodically in several places over the valley. East of the agricultural fields is a sandy, desert creosote bush (*Larrea tridentata*) community which serves as the wildlife study plot near East Mesa.

In Imperial Valley, geothermal development is more advanced than at Roosevelt Hot Springs. Drilling has occurred at several locations including, Heber, Brawley, East Mesa, Westmoreland and a test facility has been constructed at Niland which is investigating the use of concentrated brines.

Roosevelt Hot Springs is in southwest Utah on land managed largely for grazing cattle and sheep, although on irrigated acreage, at considerable distances from the geothermal resource activity, alfalfa, corn, potatoes and cereal crops are produced. There are also some dairy associations in the Minersville area. Water availability for range animals is a problem and some grazing allotments must have additional water delivered.

The 40-square-mile geothermal development site ranges in elevation from about 5200 to 7700 feet northeast of Milford on the western side of the Mineral Mountain Range. These mountains, extending in a north-south direction, not only are the dominant geographic feature but also have an obvious effect on the watershed and climatic conditions within the study area. Sloping foothills west of the mountains extend out toward the relatively flat Escondido Desert. The geothermal development at Roosevelt Hot Springs is in the exploratory stage. Some test wells have been drilled but no power plants have been constructed.

There are four basic vegetative communities within the Utah geothermal study area (personal communication K. Brown). Dominant plant species for the two major communities are the black sage (*Artemisia nova*) and the pinyon-juniper association (*Pinus edulis* and *Juniperus osteosperma*). Relatively deep sandy soil along a large wash supports big sagebrush (*Artemisia tridentata*)

and big rabbit brush (*Chrysothamnus nauseosus*) communities. In addition to these four major plant groupings, there is a small restricted greasewood community (*Sarcobatus vermiculatus*) within the study area. The overall area is basically semiarid with an annual precipitation of approximately eight inches and with large annual and daily variations in temperature. However, localized thunderstorms can be quite severe, and the annual precipitation generally increases with increasing elevation.

At both study areas, the wells that have been drilled are being tested prior to completion of commercial plant facilities.

The effluent being extracted during these activities contains many chemical elements. The main mineral constituents are the bicarbonates, carbonates, sulfates and dichlorides of sodium, potassium, magnesium and calcium. The proportions of these minerals vary with the location and depth of the wells, where the water also contains varying amounts of less common elements, some of them at levels that cause environmental concern. Table 2 presents data on the concentration of various elements in geothermal brine for Roosevelt Hot Springs and Imperial Valley sites.

Chemical elements tend to circulate through characteristic pathways within an ecosystem and travel between the abiotic component and the living organisms. The ecosystem, as a functional unit, has evolved in such a way as to remain viable and stable within the context of these cycles. However, human activities greatly accelerate the movement of many elements, and the cycles can become overloaded or irregular, changes that are obviously important for biologically essential elements but less apparently critical, but of concern, for non-essential elements if there is an immediate or long-term detrimental effect.

TRACE ELEMENT FINDINGS

Some of the 1977 collection-year tissue assays have been completed for cattle blood and for selected wildlife species. Elemental concentrations in the whole blood of Imperial Valley cattle are shown in Table 3. Concentrations in the carcass, washed pelt, unwashed gastrointestinal tract and whole body (extrapolated) are shown in Table 4 for kangaroo rats captured at Roosevelt Hot Springs. Percentages of the total body amounts found in each of these divisions are shown in Table 5 and some comparisons in dry weight concentration between kangaroo rats and laboratory rats appear in Table 6. The elemental concentrations in liver, kidney and hair tissue from Great Basin pocket mice collected at Roosevelt Hot Springs are shown in Table 7. All analytical data are considered preliminary and will be compared with the results from ongoing assays, future collections, analysis and evaluation of quality assurance data.

The information will be summarized ultimately as baseline concentrations for the sentinel animals. It should be pointed out, however, that, while blood is relatively easy to collect, it is affected by a variety of homeostatic mechanisms, and elemental concentrations in it may tend to remain constant. This homeostasis permits the body's internal environment to remain constant

TABLE 2. CONCENTRATIONS OF VARIOUS ELEMENTS IN GEOTHERMAL FLUIDS
AT ROOSEVELT HOT SPRINGS AND IMPERIAL VALLEY

Element	Roosevelt Hot Springs ($\mu\text{g/ml}$)*	Niland California ($\mu\text{g/g}$)**	Imperial Valley, Calif. "worst case composite" ($\mu\text{g/g}$)**
Aluminum	0.32	-	-
Arsenic	-	-	15.0
Barium	trace***	250.0	570.0
Boron	-	390.0	-
Chromium	trace	-	1.80
Cobalt	trace	-	0.40
Copper	trace	-	10.0
Iron	7.2	2000.0	4200.0
Lead	0.5	80.0	400.0
Silicon	150.0	-	-
Strontium	0.8	440.0	740.0
Tin	trace	-	0.65
Zinc	0.1	500.0	970.0

* In-house analysis of one site by optical emission

** From Schieler, 1976, compiled from several sources

*** "trace" is less than 0.1 $\mu\text{g/ml}$

Table 3. ELEMENTAL CONCENTRATIONS IN WHOLE BLOOD OF IMPERIAL VALLEY CATTLE DURING MAY 1977 (Mean values expressed in $\mu\text{g/g}$ wet weight; standard deviation and ranges are also shown).

Element	13 Adult Feedlot Animals	20 New- Arrival Calves
Zinc	3.82 ± 0.9 (2.64 - 6.25)	3.92 ± 0.5 (2.74 - 5.18)
Copper	1.16 ± 0.2 (0.80 - 1.46)	1.04 ± 0.3 (0.68 - 1.92)
Iron	457.90 ± 75.1 (312.62 - 558.64)	345.16 ± 46.8 (237.96 - 421.11)
Aluminum	1.20 ± 0.2 (0.93 - 1.52)	1.06 ± 0.5 (0.74 - 3.08)
Boron	0.52 ± 0.1 (0.33 - 0.68)	0.46 ± 0.1 (0.21 - 0.65)
Silicon	7.81 ± 1.1 (6.06 - 9.33)	7.04 ± 1.6 (4.92 - 12.08)
Strontium	0.21 ± 0.0 (0.18 - 0.28)	0.25 ± 0.0 (0.18 - 0.32)
Barium	0.70 ± 0.1 (0.56 - 0.92)	0.64 ± 0.2 (0.31 - 1.06)
Cadmium	$0.74 \pm 0.1^*$ (0.67 - 0.82)	0.58^{**}

* Values based on three animals, others $<3.0 \mu\text{g/g}$ dry weight
 ** Value for one animal, others $<3.0 \mu\text{g/g}$ dry weight
 Concentrations for lead were $<1.0 \mu\text{g/g}$ dry weight

Table 4. ELEMENTAL CONCENTRATIONS IN CARCASS, PELT, AND GASTROINTESTINAL TRACT FROM KANGAROO RATS (*Dipodomys ordii*) COLLECTED AT ROOSEVELT HOT SPRINGS DURING MAY 1977 (Mean values expressed in $\mu\text{g/g}$ wet weight; standard deviations and ranges are also shown).

Element	Carcass	Pelt	Unwashed G.I. Tract	Extrapolated Whole Body
Zinc	42.61 $\pm$ 11.6 (28.86 - 38.36)	10.18 $\pm$ 2.4 (7.63 - 14.73)	42.48 $\pm$ 13.5 (25.77 - 68.28)	35.96 $\pm$ 8.3 (26.94 - 45.34)
Copper	2.47 $\pm$ 0.3 (2.07 - 3.00)	3.99 $\pm$ 0.6 (3.05 - 4.85)	5.31 $\pm$ 1.4 (3.29 - 7.41)	3.05 $\pm$ 0.4 (2.41 - 3.84)
Iron	76.42 $\pm$ 9.81 (60.57 - 96.93)	136.65 $\pm$ 36.1 (60.89 - 184.01)	116.12 $\pm$ 54.3 (60.00 - 184.12)	91.75 $\pm$ 13.8 (75.91 - 118.86)
Aluminum	5.15 $\pm$ 1.5 (3.59 - 8.17)	103.03 $\pm$ 36.1 (43.63 - 163.52)	32.72 $\pm$ 24.4 (7.05 - 65.00)	27.17 $\pm$ 9.3 (16.49 - 43.92)
Silica	11.47 $\pm$ 3.0 (6.83 - 14.63)	202.79 $\pm$ 75.8 (76.80 - 332.29)	254.44 $\pm$ 231.5 (77.48 - 874.76)	71.06 $\pm$ 28.8 (47.72 - 132.81)
Strontium	12.80 $\pm$ 4.9 (4.79 - 19.76)	3.31 $\pm$ 2.6 (1.24 - 10.19)	2.32 $\pm$ 1.1 (0.93 - 3.89)	9.98 $\pm$ 3.4 (4.18 - 15.07)
Barium	4.26 $\pm$ 0.8 (2.74 - 5.43)	1.94 $\pm$ 0.9 (0.73 - 4.21)	3.75 $\pm$ 1.4 (2.31 - 7.15)	3.75 $\pm$ 0.7 (2.56 - 4.64)

Cont. Table 4. ELEMENTAL CONCENTRATIONS IN CARCASS, PELT, AND GASTROINTESTINAL TRACT FROM KANGAROO RATS (*Dipodomys ordii*) COLLECTED AT ROOSEVELT HOT SPRINGS DURING MAY 1977 (Mean values expressed in $\mu\text{g/g}$ wet weight; standard deviations and ranges are also shown).

Element	Carcass	Pelt	Unwashed G.I. Tract	Extrapolated Whole Body
Lead	*	1.19 $\pm$ 0.9 (0.51 - 2.53)	*	0.24 $\pm$ 0.2** (0.10 - 0.54)
Cadmium	***	*	***	***
Magnesium	648.66 $\pm$ 89.2 (475.59 - 775.05)	112.28 $\pm$ 40.0 (75.31 - 217.68)	778.15 $\pm$ 225.3 (500.44 - 1142.97)	543.72 $\pm$ 71.4 (399.39 - 640.13)
Manganese	0.58 $\pm$ 0.2 (0.37 - 0.94)	1.86 $\pm$ 0.7 (1.06 - 3.27)	29.20 $\pm$ 10.3 (18.65 - 47.89)	3.37 $\pm$ 1.1 (1.73 - 4.57)
Boron	**** -	2.55 $\pm$ 4.2 (0.15 - 14.40)	1.13 $\pm$ 0.4 (0.62 - 2.00)	0.605 $\pm$ 0.8 (0.10 - 2.90)

* Undetectable

** Values for four animals

*** Concentration $< 3.0 \mu\text{g/g}$ dry weight

**** Concentration $< 2.0 \mu\text{g/g}$ dry weight

Table 5. APPROXIMATE DISTRIBUTION OF VARIOUS ELEMENTS IN KANGAROO RATS CAPTURED AT ROOSEVELT HOT SPRINGS DURING 1977 (Amounts expressed as percentages of the whole body total amounts in carcass, pelt and gastrointestinal tract)

Element	Carcass	Pelt	Unwashed G.I. Tract
Aluminum	14	76	10
Silicon	12	60	28
Barium	81	10	9
Iron	60	29	11
Zinc	83	6	11
Boron	-	70	30

Table 6. PRELIMINARY COMPARISONS BETWEEN THE WHOLE BODY ELEMENTAL CONCENTRATIONS FOR KANGAROO RATS AND LABORATORY RATS (Mean values expressed in $\mu\text{g/g}$ of dry weight $\pm$ two standard errors)

Animal	Zinc	Aluminum	Strontium	Boron
Kangaroo Rats	112.65 $\pm$ 20.9	84.42 $\pm$ 19.5	31.44 $\pm$ 7.9	11.65 $\pm$ 1.6
Laboratory Rats	86.67 $\pm$ 6.5	69.88 $\pm$ 6.2	4.94 $\pm$ 0.6	3.07 $\pm$ 0.4

Table 7. PRELIMINARY DATA ON ELEMENTAL CONCENTRATIONS IN LIVER, KIDNEY AND HAIR FROM GREAT BASIN POCKET MICE (*Perognathus parvus*) COLLECTED AT ROOSEVELT HOT SPRINGS DURING 1977 (Mean values expressed in $\mu\text{g/g}$ wet weight for liver and kidney and in $\mu\text{g/g}$ dry weight for hair; standard deviations and ranges are also shown).

Element	Liver	Kidney	Hair
Zinc	17.27 ± 4.9 (8.89 - 23.64)	9.75 ± 4.8 (4.66 - 19.91)	67.36 ± 58.7 (16.15 - 162.50)
Copper	4.83 ± 0.8 (3.41 - 5.45)	5.44 ± 2.1 (2.04 - 9.41)	19.84 ± 4.0 (15.20 - 25.10)
Boron	6.26 ± 4.0 (0.09 - 12.73)	9.36 ± 5.9 (2.03 - 25.63)	21.46 ± 10.2 (10.10 - 33.20)
Aluminum	22.46 ± 22.9 (8.51 - 56.62)	12.12 ± 12.5 (1.56 - 39.45)	852.00 ± 584.6 (237.50 - 1700.00)
Strontium	0.61 ± 0.2 (0.31 - 0.84)	0.47 ± 0.3 (0.17 - 1.30)	13.14 ± 9.9 (2.00 - 28.30)
Lead	*	*	2.93 ± 0.7 (2.20 - 3.60)
Cadmium	**	**	**

* Undetectable

** Values $<3.0 \mu\text{g/g}$ dry weight

at all times. For example, during periods of shortages of needed requirements, a combination of factors such as increased assimilation, restricted excretion and/or release of material from storage sites allows the blood and the tissues to maintain normal physiological levels. Opposite phenomena occur during periods of excess intake: less assimilation, increased excretion and shunting of material to storage sites.

Homeostatic control routes by which ruminants adapt to varying intakes of calcium, magnesium, sodium, potassium, chlorine, iron, zinc, copper, iodine, cobalt, manganese, molybdenum, selenium, fluorine, nickel and cadmium have been summarized by Miller, 1975. These control routes include changes in the percentage of elemental uptake from the gastrointestinal tract, in the amount of an element excreted and in the elemental deposition in certain tissues. In the case of hair, elemental components have been sequestered from the metabolic processes and therefore are not part of a fluctuating elemental pool. Furthermore, the keratinous (fibrous protein) outer structure of each hair is resistant to chemical alteration. Results from hair analysis will be used to establish baseline concentrations and, in the case of rodent hair, to determine whether the elemental concentrations in hair reflect elemental concentration in the body organs. Hair has been reported to reflect environmental exposure and bodily stores (Hammer et al., 1971; Corridan, 1974; Hopp, 1976), although others (Huckabee et al., 1972) have not supported this correlation. It is hoped that further analyses will determine if blood, hair and other organs are feasible as indicators of environmental exposure.

An essential part of evaluating elemental tissue levels is an understanding of what is known about selected elements of interests (i.e., absorption, distribution, retention etc.). At present the elements of major interest for this study are aluminum, arsenic, boron, cadmium, lead, strontium and zinc.

Aluminum has not been shown to be an essential element although Underwood (1977) speculated that it may be involved in cellular energy metabolism. It is poorly absorbed, so that a two-fold increase in oral exposure does not significantly affect retention (Underwood, 1977), and it is excreted mainly in the feces. In humans the highest levels are found in the lung and skin, and it is completely turned over after 30 days (Tipton and Cook, 1963; Tipton et al., 1966). Wild rats absorbed 38% of ingested aluminum (Kaufman et al., 1976). When high doses of aluminum are given to rats (200 mg/kg), excretion via urine increased and body retention was mainly in the liver, testes and bone (Underwood, 1977).

Arsenic, a non-essential, toxic element is widely distributed in the body (Underwood, 1977). Body retention varies based on chemical form. Rats retained 11.4% of naturally occurring arsenic, 14.8% of AsO_3 and 18.9% of As_2O_5 (Coulson et al., 1935; Morgareidge, 1963). Hair and nails are select places for arsenic determinations since they serve as major deposition sites (26% and 15%, respectively, of total body burden), but they also act as excretory routes and may not reflect body stores (Peoples et al., 1975); however, they do accumulate the element over long periods of exposure (Shapiro, 1967). Other storage sites include liver, heart, skin and spleen (Peoples,

1964, 1975). Absorbed arsenic is excreted at the rate of 74.6%, mainly via urine (Coulson et al., 1935).

Boron is an essential element for higher plants but not for animals (Underwood, 1977). Boron is found throughout the human body in levels between 0.5 to 1.5 $\mu\text{g/g}$ dry weight with highest levels in bones. Ingestions of large amounts of boron (boric acid) reportedly leads to increased levels in the brain (Underwood, 1977). In humans, it was found that over 30 days boron was more than completely turned over (Tipton et al., 1966). In normal adults the major sites of deposition were found to be the air passages, brain and liver (Tipton and Cook, 1963).

Cadmium is a non-essential toxic element, which lacks an effective homeostatic control. It has a reported half-life of 33 years in humans (Underwood, 1977). Intestinal absorption varies slightly between species, but most studies indicate assimilation of 2%-10% of a given dose (Friberg et al., 1971; Fleischer et al., 1974), with an average of 6% (Fassett, 1975). This is in contrast to 40% reported for respiratory retention (Fassett, 1975). Retention of cadmium has been estimated at 3%-8% over a 50-year period for humans (Friberg et al., 1971) and as low as 1% over 1 year for rats (Fleischer et al., 1974). Two-thirds of cadmium absorbed is found in the liver and kidney (Underwood, 1977), and some is deposited in hair. Excretion is mainly via the feces while urinary excretion accounts for only about 1% of absorbed dose (Friberg et al., 1971).

Lead, another non-essential, toxic element is absorbed at different rates based on speciation and age: sheep and rabbits 1.3% of oral dose (Baxter, 1950), adult rats 4.7% (Forbes and Reina, 1972), and man 5%-10% (Underwood, 1977). In humans 90% is retained in the skeleton, but high lead intake raises levels in the liver, kidney and hair. Lead is excreted slowly via bile and feces. In cattle the excretion is 91%-97% in the feces and 1.3%-2.4% in the urine (Underwood, 1977).

Strontium absorption varies with animal species: wild rats 33% of digested ash free food (Kaufman et al., 1976), while rabbits absorbed 13.5% of oral dose (Lloyd, 1967). The average absorption rate in animals is 5%-25% (Underwood, 1977), and 99% of retained strontium is found in the bone (Underwood, 1977). The strontium deposited in adult soft tissues is concentrated in the intestinal tract, aorta and larynx (Tipton and Cook, 1963). Absorbed strontium is excreted mainly via urine.

Zinc is an essential trace element in animals as part of many metallo-enzymes. Its assimilation and excretion is homeostatically controlled. The uptake of zinc varies from 34% of digested ash free food in wild rodents to 30% of dose in cattle (Miller, 1969; Kaufman, 1976). In cattle, 40% of orally administered ^{65}Zn was retained after 10 days (Miller, 1969). The highest levels of zinc are found in the hair, bone, skin, liver and kidney (Miller, 1969; Underwood, 1977). Zinc is excreted (70%) via the feces (Underwood, 1977).

CENSUS FINDINGS

Monitoring the population parameters of small mammals has been added to this study to include some indication of ecosystem stability. Characteristics to be studied include food preferences, habitat association, species composition, and abundance. Rodents were selected for primary emphasis in the census due to their expected abundance, ease of collection and importance in the food web (food source for snakes, hawks, owls, coyotes, foxes, etc.) as well as their own particular food preferences (Table 8). Related studies are in progress on the vegetative characteristics at Roosevelt Hot Springs. Most studies involving censusing (estimate of abundance) utilize removal, non-removal or non-trapping (e.g. direct observation) techniques. Non-removal trapping, involving a mark and release procedure, was selected to accomplish the study objectives (Lincoln, 1930; Hayne, 1949). This approach assumes that every marked animal will become randomly re-distributed in the population and will have the same probability of future capture as an unmarked (not previously captured) animal. The method further assumes that, during an individual trapping effort, dramatic changes (death, migration, immigration, etc.) are not occurring.

Some changes in the animal population probably will be noted that are unrelated to the geothermal operation. Even if the species within a community remain relatively stable, their absolute numbers frequently will fluctuate. Cyclic fluctuations can result from seasonal reproductive patterns that themselves are not identical from year to year. Furthermore, long-term fluctuations will be virtually impossible to predict during this study period, and no effort is currently being made to estimate potential changes in the occurrence or transmission of pathogens, changes in parasitic infestation or potential hazards to long-term reproductive success.

Relative abundance determinations for lagomorphs have indicated a population decrease from June to July (Table 9). The majority of animals sighted were blacktailed jackrabbits (*Lepus californicus*). Unless a relationship between roadside populations and the general population can be established, these data can be used only to reflect fluctuations from one census period to another. Estimating rabbit and hare populations by the roadside count technique has been a common census method (Lewis, 1970), although livetrapping (Edwards and Eberhardt, 1967) and pellet plots to establish indirect counts (Adams, 1959) have been employed under various conditions.

Both rodent census sites at Roosevelt Hot Springs were located along the margins of pinyon-juniper communities that, to some extent, represented vegetative transition zones. Table 10 presents relative abundance data for rodents associated with sagebrush and sagebrush-juniper communities as well as a composite value for animals captured in both plant groupings.

The numerical decrease noted for July might suggest a seasonal trend but these limited observations do not allow for a definitive statement. Collections made using the census line approach will provide the relative information but not a density estimate (number of animal per unit area). To determine density, the effective trapping area must be known, and this area

Table 8. POTENTIAL FOOD PREFERENCES OF SELECTED MAMMALS
POSSIBLY OCCURRING AT ROOSEVELT HOT SPRINGS

Animals	Percentage of Total Diet			
	Seeds	Vegetation	Insects	Vertebrates
Blacktailed Jackrabbit <i>Lepus californicus</i>	1.0	99.0	-	-
Antelope Ground Squirrel <i>Ammospermophilus leucurus</i>	45.5	32.0	11.5	7.5
Desert Woodrat <i>Neotoma lepida</i>	1.0	98.8	trace	-
Merriam's Kangaroo Rat <i>Dipodomys merriami</i>	82.0	16.0	2.0	-
Long-tailed Pocket Mouse <i>Perognathus formosus</i>	82.9	16.1	1.2	-
Little Pocket Mouse <i>Perognathus longimembris</i>	85.6	12.9	1.3	-
Deer Mouse <i>Peromyscus maniculatus</i>	70.0	9.0	21.0	-
Brush Mouse <i>Peromyscus boylii</i>	82.2	11.2	6.5	-
Pinyon Mouse <i>Peromyscus truei</i>	56.1	25.9	17.4	-
Pinyon Mouse <i>Peromyscus truei</i>	56.1	25.9	17.4	-

Values, based on the yearly average of stomach contents, were taken from reports on Nevada, Wyoming or Arizona mammals (Williams, 1959; Hayden, 1966; Bradley, 1968; Bradley and Mauer, 1971; Nelson et al., 1975).

Table 9. NUMBER OF RABBITS/HARES COUNTED AT ROOSEVELT HOT SPRINGS DURING JUNE AND JULY 1977

Month	Census Area	Number of animals per km travelled
June	Test Area	2.03
	Distance Control	-
July	Test Area	0.92
	Distance Control	1.40

Table 10. RELATIVE ABUNDANCE OF RODENTS AT ROOSEVELT HOT SPRINGS DURING JUNE AND JULY 1977

Month	Collection Area	Vegetative Association	Relative* Abundance
June	Test Area	Sagebrush	17.50
		Sagebrush/Juniper	24.00
		Total	39.00
	Distance Control	Sagebrush	16.70
		Sagebrush/Juniper	23.40
		Total	40.30
July	Test Area	Sagebrush	12.00
		Sagebrush/Juniper	10.50
		Total	21.67
	Distance Control	Sagebrush	8.75
		Sagebrush/Juniper	24.00
		Total	32.45

* Expressed as $P = SM/R$, where P = total population, S = number of unmarked and marked animals captured on the last day, M = total number of animals marked and released, R = number of recaptures occurring on last day (Lincoln, 1930).

is usually based on some multiple of the trap spacing or home ranges of individual species (Jorgensen and Hayward, 1965; Smith et al., 1975). A number of trapping techniques are available for this purpose (Smith et al., 1971; Smith et al., 1972; and O'Farrell et al., 1977). We are currently utilizing a square grid configuration (a series of parallel lines of traps spaced 15 m apart). The wooden staked grid patterns have been positioned at the two Roosevelt Hot Springs collection sites and future population estimates will be reported as the number of animals per hectare. Trapping techniques, density and the associated problems in assessing small mammal populations have been reviewed by many investigators (Smith et al., 1975). It should be noted that for comparison purposes variables such as the trapping configuration (trap spacing), trap type and response, bait, duration of the census, season of collection, climatic conditions, overall habitat characteristics, as well as the census calculations methods, must all be considered.

Associated with population studies, identifications of several mammalian wildlife species were made at Roosevelt Hot Springs during 1977 and verified utilizing Fautin (1946) and Hall and Kelson (1959). The species included: the blacktailed jackrabbit (*Lepus californicus*), Nuttall's cottontail (*Sylvilagus nuttalli*), Desert cottontail (*Sylvilagus audubonii*), rock squirrel (*Spermophilus variegatus*) antelope ground squirrel (*Ammospermophilus leucurus*), least chipmunk (*Eutamias minimus*), Great Basin pocket mouse (*Perognathus parvus*), Ord's kangaroo rat (*Dipodomys ordii*), western harvest mouse (*Reithrodontomys megalotis*), canyon mouse (*Peromyscus crinitus*), deer mouse (*Peromyscus maniculatus*), brush mouse (*Peromyscus boylii*), desert woodrat (*Neotoma lepida*), western big-eared bat (*Plecotus townsendii*), coyote (*Canis latrans*), long-tailed weasel (*Mustela frenata*), Badger (*Taxidea taxus*) and mule deer (*Odocoileus hemionus*).

Preliminary data on species composition, combining both live trap and snap trap collections, indicate that the Great Basin pocket mice were the predominant rodent for Roosevelt Hot Springs trapping areas accounting for 69.8% of the captures followed by: deer mice, 14.3%; Ord's kangaroo rats, 11.1%; western harvest mice, 2.4%; desert woodrats, 1.6%; and canyon mice, 0.8%. Numerical relationships between species (i.e., composition and/or diversity) may be more useful to the program than a population assessment since changes in species indices are used frequently in pollution ecology to measure the stress in an ecosystem (Beijer and Jernelov, 1928).

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APPENDIX A

SAMPLE PREPARATION FOR TRACE ELEMENT ANALYSIS BY ATOMIC ABSORPTION SPECTROSCOPY

A. Laboratory Equipment, Reagents and Personnel.

1. Equipment

- a. Sample holding and storage containers (jars, bags, vials, etc.) should be made of polyethylene or a similar inert plastic.
- b. All other items used in sampling and sample handling (surgical instruments, spatulas, forceps, tongs, etc.) should be either Teflon-coated or made of plastic or stainless steel.
- c. Flasks or beakers used during microwave digestion should be made of Teflon or of a high-grade borosilicate glass. These containers should be used for either high- or low-level samples only, depending on previous use, and be so marked.
- d. Containers, flasks and instruments are cleaned prior to use. Wash in warm, detergent-supplemented water and rinse with deionized water. Soak for at least four hours in 1:1 conc. $\text{HNO}_3/\text{H}_2\text{O}(\text{V/V})$ or in 1:1:2 $\text{HNO}_3/\text{HCL}/\text{H}_2\text{O}(\text{V/V})$. Rinse with deionized water and oven dry at 50-60°C. Cover washed and dried equipment until needed.
- e. Dissecting tables are cleaned with dilute nitric acid and covered with disposable plastic lined pad. Fresh pads and gloves as well as fresh (cleaned) sets of instruments are used for each animal.

2. Reagents

- a. All water used must be deionized.
- b. Reagent impurity concentrations must be low enough that they do not interfere with elements of interest.

3. Personnel

- a. Persons involved in either washing operations or in tissue sampling will wear laboratory coats and sterile surgical gloves.
- b. All persons involved in sample digestion must wear rubber aprons, rubber gloves and safety glasses (or preferably safety masks).
- c. Samples, glassware, instruments, etc., should never be touched with bare hands. Gloves are always worn and cleaned tongs or forceps are used. Smoking is not allowed in the laboratory.
- d. All personnel involved in any aspect of sampling, sample handling, or sample preparation must be thoroughly familiar with the study procedures and objectives as well as difficulties associated with trace element work.

B. Tissue Collection, Preparation and Storage.

1. General Procedures

- a. Clean, label, cap and weigh sample containers and assemble materials needed for dissection.
- b. Perform dissection and place isolated tissues in respectively labeled containers. Cap and weigh containers recording net tissue wet weight.
- c. Place open containers in drying oven (60°C) and dry to constant weights. Close containers, record sample dry weight and store containers (with sample) in freezer.

Note: Freeze drying is the preferred drying method as potential for contamination is usually reduced at lower temperatures. Freeze drying is used whenever equipment is available.

2. Procedures Applicable to Blood, Hair and Egg Samples.

- a. The following procedure applies to blood samples.
 - (1) Samples are collected in sterile heparinized syringes (or vacutainer tubes) with sterile needles. Syringes

are emptied into labeled, weighed polyethylene vials.

- (2) Weighed samples are stored in the freezer.
- b. The following procedure applies to hair samples. (Modified from Clarke and Wilson, 1974).
- (1) To remove surface contamination, swirl hair sample (using forceps) in a hot detergent solution (10 ml commercial detergent in 600 ml of deionized water) and rinse in distilled water. Wrap sample in a 6 x 12" piece of cheesecloth, fold the ends over and secure them with white adhesive tape. Write identification number on the tape with an indelible marker.
 - (2) Slightly agitate the sample in deionized water and wring it out. Place sample in a hot detergent solution (10 ml detergent + 600 ml deionized water), squeeze it repeatedly with a glass rod and wring out.

Repeat step (2).
 - (3) Rinse sample in deionized water and wring it out. Place sample in acetone (300 ml), agitate and squeeze it repeatedly with a glass rod and then wring out.
 - (4) Place sample in 600 ml of a hot, filtered, saturated solution of ethylenediaminetetraacetic acid (EDTA) in deionized water, agitate and squeeze with a glass rod. After 5 minutes, remove and wring out the sample, wash sample twice in deionized water and wring out thoroughly.
 - (5) Dry sample at 60°C, open cheesecloth and transfer sample to a preweighed, labeled polyethylene container. Record net weight of clean hair sample and store in freezer.

c. The following procedure applies to egg samples.

- (1) Wash intact eggs with a detergent solution and with deionized water.
- (2) Break individual eggs and separate into yolk, white and shell. Transfer each fraction to respective preweighed, labeled polyethylene containers. Record wet weight and store sealed containers in the freezer.

Note: Egg yolk may contain a high fat content and an extraction process must be employed to remove lipids.

C. Microwave Assisted Digestion of Biological Samples.

Note: Personnel conducting sample digestions must wear rubber gloves, apron and safety goggles or preferably safety masks. Accurate records of digestion steps are essential.

1. Sample Size

- a. Sample size for microwave digestion should not exceed 1 g dry weight or, in the case of whole blood, should not exceed 5 g wet weight.
- b. Samples should be treated with a minimum quantity of the purest acid available. Recommended nitric acid additions are 10 ml per 0.5 g of dry tissue or per 2.5 g of whole blood.
- c. Volumetric flask size will be determined by initial sample size or requirements for replicate analyses.

2. Reagents

- a. Concentrated nitric acid is transferred to a container (borosilicate) of convenient size and placed in hood.
- b. Acid container labels must indicate preparation date and container contents. Do not allow dust to accumulate on the exterior of the container.

3. Digestion Procedure

- a. Transfer approximately 0.5 g of dried tissue to a preweighed 100 ml Teflon beaker or to a 125 ml Erlenmeyer flask. Record tissue weight.
- b. Add 10 ml conc. nitric acid to sample, swirl and allow to stand a few minutes (starts digestion).
- c. Place beaker (containing sample) in microwave oven and turn switch to "high." When acid begins to boil, turn switch to "medium-low." As digestion progresses, digest color changes from dark to light; brown fumes are emitted. Digestion time is not critical, but is approximately one minute per ml of acid used. As digestion approaches completion the solution should be clear or straw-colored and transparent. The digestion should be stopped when the solution volume is approximately 0.5 ml.

Note: If solution is not light colored and transparent more acid may need to be added and the digestion step repeated.

- d. Turn switch to "off." When all fumes have been removed by exhaust system, open oven door, remove samples with tongs and cover sample beaker and transfer beaker to the hood for cooling.
- e. Add 3-4 ml of deionized water to beaker, swirl and pour contents into volumetric flask. Rinse beaker with 2-3 ml of deionized water and add this to flask. Bring to volume with deionized water.

Note: If precipitate remains in flask, add HCl or another suitable solvent dropwise (not to exceed 5.0% of volume) until precipitate is dissolved. Water is then added to volume.

- f. Transfer solution from volumetric flask to a polyethylene container for storage. Seal the labeled container and place it in the freezer.

D. Potential Errors During Sample Preparation and Storage.

1. Low Values

- a. Incomplete sample digestion may result in incomplete solubilization.

- b. Overheating of sample during digestion may result in partial volatilization of certain elements (e.g., mercury, arsenic).
- c. Careless manipulation of samples and sample solutions may result in spillage.
- d. Under certain conditions, elements of interest may be absorbed to container wall or to precipitates formed during digestion or storage.
- e. During sample storage period, bacterial action may increase volatility of certain elements (e.g., formation of methyl mercury). This can be suppressed by freezing samples and keeping storage time to a minimum.

2. High Values

- a. Use of less-than-clean laboratory equipment and use of impure reagents will produce inaccurate values.
- b. Samples can also be contaminated by airborne dusts, mists and fumes.
- c. Partial evaporation of water from improperly sealed digest containers will concentrate the solutes.

APPENDIX B

PREPARATION OF TISSUE SAMPLES FOR OPTICAL EMISSION SPECTROSCOPY ANALYSIS

Note: All equipment used in these procedures will have been thoroughly cleaned as was previously described.

1. Large samples (muscle, pelt, carcass, gastrointestinal tract, etc.) are isolated and dried as described earlier. The dried, frozen samples are homogenized (usually 2 to 3 minutes) in a Tekmar analytical grinding mill and part of the sample submitted for analysis.
2. Small samples (rodent organs) are isolated and dried as described earlier. Since the dry weight of these small organs is usually between 0.01 and 0.8 grams, each organ is cut into small pieces and the entire sample submitted for analysis.
3. Hair samples are washed as described, dried, cut into sections 4 to 5 mm long and submitted for analysis.
4. Blood and eggs (separated into yolk and albumin) are dried, then manually powdered and mixed with a Teflon rod and submitted for analysis.